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Co-existance of Aminoglycoside Modifying Enzymes (AMEs) genes and *mec*A gene among nosocomial isolates of *Staphylococcus aureus* in Surgical Intensive Care Units in Kasr Al-Ainy hospitals, Cairo University

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Abstract

Background: In *Staphylococcus aureus* aminoglycosides and methicillin resistance are closely associated. We aimed at detection of the prevalence of methicillin and aminoglycosides resistances in *S. aureus* isolated from surgical intensive care units (ICUs) in Kasr-Al-Aniy hospitals, the association of *mec*A gene and aminoglycoside modifying enzymes (AMEs) genes among these isolates and the accuracy of cefoxitin disc diffusion method in relation to PCR.

Methods: A total of 150 clinical samples were collected. Cultivation and identification of isolates were done by the standard microbiological techniques. All *S. aureus* isolates were tested for methicillin and aminoglycosides resistance by disc diffusion method and for presence of *mec*A gene and AMEs genes by multiplex-PCR.

Results: A high rates of *mec*A gene (89.58%) and AME genes (60.42%) were found in 48 *S. aureus* isolates (32%). Co-existence of *mec*A gene with one or more of AME genes was detected in 60% of the isolates. The *mec*A gene was detected in 87% of cefoxitin resistant isolates and *aac(6')-Ie+aph(2'')* was the most predominant AME genes.

Conclusion: This study shows wide spread of *mec*A gene in association with aminoglycoside modifying enzymes in the ICUs of Kasr-Al-Aniy hospitals in Cairo.

Keywords: *S. aureus* - *mec*A gene - aminoglycoside modifying enzymes genes -PCR

Introduction

Staphylococcus aureus (S. aureus) , in particular, methicillin-resistant *S. aureus* (MRSA) has long been responsible for a great deal of human morbidity and mortality. MRSA are increasingly important causes of hospital and community acquired infections throughout the world, and most hospital infections caused by *S. aureus* due to central venous catheter associated bacteraemia, lower respiratory tract infection, skin and soft tissue infection as well as ventilator associated pneumonia (1, 2).

Multidrug resistance is becoming prevalent among *Staphylococcus aureus* especially those isolated from intensive care units and from blood cultures worldwide (1). Inevitably this has left fewer effective antibiotics to treat these often life-

threatening infections. As rapidly as new antibiotics are introduced, staphylococci are developing efficient mechanisms to neutralize them (3).

Because the aminoglycosides have a rapid and intense killing power, they have been commonly administered along with the glycopeptides in treatment of multidrug-resistant (MDR) *S. aureus* infections (4). Unlike most antibiotic inhibitors of protein synthesis in bacteria that lead to bacteriostasis, the aminoglycosides are potent bactericidal agents that inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit and by disrupting the integrity of bacterial cell membrane (5).

Detection of *mec*A or the protein expressed by *mec*A, the PBP 2a or PBP 2', are the most accurate methods for prediction of resistance to oxacillin and can be used to confirm results for isolates of staphylococci from serious infections. Isolates of staphylococci that carry the *mec*A gene, or that produce PBP 2a should be reported as oxacillin resistant, and those isolates that do not carry *mec*A or do not produce PBP2a should be reported as oxacillin susceptible (6).

It is reported that *S. aureus* resistance to aminoglycosides is closely associated with methicillin resistance (7). The main mechanism of aminoglycoside resistance in staphylococci is drug inactivation by plasmid- or transposon-mediated cellular aminoglycoside-modifying enzymes (AMEs) (8). These enzymes are of particular significance among staphylococci since they modify and thereby inactivate the aminoglycosides. The genes encoding such modifying enzymes include *aac(6')-Ie+aph(2'')*, *aph(3')-IIIa* and *ant(4')-Ia* (9).

Susceptibility testing for individual aminoglycoside antibiotics is usually performed in clinical microbiology laboratories by disc diffusion or measurement of the minimal inhibitory concentration (MIC) of the antibiotic (10). However, such resistance does not discriminate between the types of enzymes, which might be present in a bacterial isolate (11). This limitation has been addressed by the use of genotypic methods including dot blot hybridization and polymerase chain reaction (PCR) for detecting the presence of genes encoding AMEs and specifying their types from *Staphylococci* (7).

The aim of this work was to investigate the prevalence of methicillin-resistant and aminoglycoside resistant *Staphylococcus aureus* in surgical ICUs in Kasr-Al-Aniy hospitals and the association of *mec*A gene and AMEs genes among nosocomial isolates of *S. aureus*, in addition, to test the accuracy of cefoxitin disc diffusion method in relation to PCR.

Materials and Methods

This study was conducted on 150 patients from the general surgery, orthopedic and neurosurgery intensive care units (I.C.Us) who were admitted to adult trauma and surgical patients in Kasr Al-Ainy hospitals, Cairo University. Ninety six males and 54 females with age between 24-59 years were included in the study. All laboratory tests were carried out in the department of Microbiology and Immunology, Faculty of Medicine, Cairo University during the period from March, 2011 to September, 2012.

Sample collection

A total of 150 clinical samples of swabs, suction catheters and syringes were collected from 150 patients including 8 blood samples, 69 pus samples obtained from the floor and wall of abscesses after pus evacuation and surgical site infections from burn sites, 20 sputum samples, 32 endotracheal aspirate samples, one pleural aspirate sample and 20 catheter specimens of urine . All specimens were labeled with the date, patient's name and patient's number, time of collection and specimen type, then transported immediately to the microbiology laboratory.

Cultivation of the specimens

All specimens other than blood were cultured onto nutrient agar and blood agar plates (Oxoid Hampshire, England) and were incubated aerobically at 35°C 24 – 48 hours. Blood samples were inoculated into blood culture bottles (Oxoid , England) and mixed with the medium, then incubated aerobically at 35°C. Subcultures were done on blood agar plates every 2 days, and were incubated aerobically at 35°C 24 – 48 hours to detect bacterial growth. The blood culture bottles were considered negative after 14 days if no growth on the subcultures was detected.

Identification of the isolates

Staphylococcus aureus isolates were identified by colony morphology, Gram stained smear, catalase test and coagulase test (Plasma Coagulases EDTA Selectavial, MAST, USA).

Detection of methicillin and aminoglycoside resistance among *S. aureus* isolates by disc diffusion method:

All *S. aureus* isolates were inoculated on Mueller-Hinton agar with 2% NaCl to test for oxacillin susceptibility, while Mueller-Hinton agar was used for the other antibiotics. All inoculated plates were incubated aerobically at 35°C for 24 hours. The following antibiotic discs were used: oxacillin (OX) 1µg,

cefoxitin (FOX) 30µg, gentamicin (CN) 10µg, amikacin (A) 30µg, kanamycin (K) 30µg, netilmicin (NET) 30µg and tobramycin (T) 10µg. Inhibition zones of antibiotics were interpreted according to the CLSI guidelines (6).

Genotypic detection of methicillin and aminoglycoside modifying enzyme resistance genes in *S. aureus* isolates using the multiplex-PCR method

DNA Extraction from *S. aureus* isolates cultures was done using Biospin Bacteria Genomic DNA extraction kit (BioFlux corporation, Tokyo, Japan).

DNA amplification by Multiplex PCR was done using four sets of lyophilized primers specific for *aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*, and *mecA* gene that were designed from a site within the nucleotide sequence of the published region of each resistance gene (12) (**table 1**), Dream Taq™ Green PCR Master Mix (2X) 200 reactions of 50µl (Thermo-Fermentas, California, USA) (ready-to use solution containing Dream Taq™ DNA polymerase, optimized Dream Taq™Green buffer, 4mµ $MgCl_2$, density reagents, 2 dyes for monitoring electrophoresis process and 0.4 mµ from each of dATPs, dCTP, dGTP and dTTP) and Biometra T-Personal thermal cycler (48 wells, Ver 09/10, Germany).

Five µl of the extracted DNA was added to 25 µl of PCR master mix, 1 µ of each primer, 12 µl nuclease free water to reach a final volume of 50µl.

The amplification process was done as follows according to the manufacturer's instructions and what was published before (12) as an initial denaturation cycle at 95 ° C for 5 minutes, 35 cycles consisted of denaturation at 95 ° C for 30 seconds, annealing at 48 ° C for 30 seconds and primer extension at 72 ° C for 30 seconds followed one cycle of final extension at 72 ° C for 10 minutes.

At the end of amplification process, the maintenance temperature was 4°C.

Detection of the amplified products: The amplified DNA products sizes were detected using 2% agarose gel electrophoresis, molecular size marker(100-1000 base pair) and were visualized by UV transilluminator (Biometra, TI)

Table 1. Specific primer sequences used in the detection of *mec*A and AME genes (12):

Target genes	Primer sequence	Amplified product size
aac(6')/ *aph*(2'')	5'-GAAGTACGCAGAAGAGA-3' 5'-ACATGGCAAGCTCTAGGA-3'	491 bp
aph(3')-IIIa	5'-AAATACCGCTGCGTA-3' 5'-CATACTCTTCCGAGCAA-3'	242 bp
ant(4')-Ia	5'-AATCGGTAGAAGCCCAA-3' 5'-GCACCTGCCATTGCTA-3'	135 bp
*mec*A	5'-CCTAGTAAAGCTCCGGAA-3' 5'-CTAGTCCATTCGGTCCA-3'	bp

Statistical Analysis

Data were statistically described in terms of frequencies (number of cases) and percentages. Comparison between the study groups was done using Chi square (χ^2) and McNemar tests. Exact test was used instead when the expected frequency is less than 5. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Bacterial growth with golden yellow pigment on nutrient agar, β-haemolysis on blood agar, Gram positive cocci in grape like clusters, positive catalase test and positive slide coagulase test was considered as *S. aureus* isolate. Nine bacterial isolates were Gram positive cocci in grape like clusters, catalase test positive, produced golden yellow pigment on nutrient agar and β-haemolysis on blood agar but they were coagulase negative by the slide coagulase test so they were examined with tube coagulase test and 6 isolates of them were positive and considered as *S. aureus* isolates. A total number of 48/150 (32%) *S. aureus* isolates from clinical samples were isolated from general surgery, neurosurgery and orthopedic ICUs. The rest of the clinical samples revealed the presence 0f 56 Gram negative bacilli (36 *Enterobacteriaceae*, 13 *Pseudomonas* and 7 *Acinetobacter*), 21 Coagulase negative staphylococci, 5 *Candida* spp and 20 clinical samples were negative. The distribution of *S. aureus* isolates in specimens were the following: 19 (39.58%) from pus, abscess, surgical wound and burn site, 15 (31.25%) were from endotracheal aspirates, 7 (14.58%) from blood, 3 (6.25%) from catheters of urine, 3 (6.25) from sputum and one (2%) from pleural aspirate.

The results of antibiotic susceptibility disc diffusion method indicated the following: 41/48 (85.42%) of *S. aureus* isolates were oxacillin resistant; 39 isolates of these oxacillin resistant strains (81.25%) were also cefoxitin resistant and 2 isolates of them (4.17%) were cefoxitin sensitive (out of a total 9 cefoxitin sensitive isolates). None of these 2 oxacillin resis-

Table 2. Correlation between cefoxitin (FOX) resistance and the presence of *mec*A gene.

P value	PCR results for *mecA*		No. of strains tested by disk diffusion with FOX
	No. of negative	No. of positive	
0.256	5	34	39 (resistant)
	0	9	9 (sensitive)
	5	43	Total = 48

tant / cefoxitin sensitive isolates were harbouring *mec*A gene. Out of the 48 *S. aureus* isolates (89.58%) were harboring *mec*A gene, 34 isolates of them were cefoxitin resistant (87% of all cefoxitin resistant *S. aureus* isolates) and 9 isolates of them were cefoxitin sensitive (100% of all cefoxitin sensitive *S. aureus* isolates). There is no statistically significance (*p* value 0.256) between phenotypic cefoxitin resistance and genotypic results (**Table 2**). The results of antibiotic susceptibility showed 29 isolates (60%) showed resistance to all aminoglycosides tested (**Table 3**). Also, 34 (87%) of MRSA isolates were resistant to gentamicin, 35 (90%) to Amikacin, 38 (97%) to Kanamycin, 28 (72%) to Netilmicin and 34 (87%) to Tobramycin (**Table 4**).

AME genes were detected in 29/48 (60.42%) of *S. aureus* isolates .The *aac(6')-Ie+aph(2'')* gene was present in 26/46 (56.5%) aminoglycoside resistant *S. aureus*, and only one *S. aureus* isolate was susceptible to aminoglycoside, while *aph(3')-IIIa* and *ant(4')-Ia* were detected in 22/48 (48%) and 1/46 (2%) of aminoglycoside resistant *S. aureus*, None of the AME genes were detected in 17 aminoglycoside resistant isolates (**table 5**). Of the 39 MRSA, 23 isolates (56%) were harbouring *aac(6')-Ie+aph(2'')* while *aph(3')-IIIa* and *ant(4')-Ia* were detected in 20 (51.28%) and 1 (2.56%) isolates, respectively. In addition, *aac(6')-Ie+aph(2'')* was present in 4 / 9 (44.44%) of MSSA isolates and *aph(3')-IIIa* was present in 2/9 MSSA (22.22%) isolates (**Table 6**).

Out 48 *S. aureus* isolates, 14 isolates (29.16%) were harboring *mec*A gene alone without any AME genes, and only 7 isolates (14.58%) were harboring *mec*A gene in combination with *aac(6')-Ie+aph(2'')* and *2* isolates (4.16%) were harboring *mec*A gene in combination with *aph(3')-IIIa*. Also, 20 isolates (41.66%) carried *mec*A gene in combination with *aac(6')-Ie+aph(2'')* and *aph(3')-IIIa* There are statistical significant correlations between *mec*A gene and *aac(6')-Ie+aph(2'')* (p value = 0. 007) and between *mec*A gene and *aph(3')-IIIa* (p value = 0. 030). All the 20 isolates that harboring *mec*A gene in combination with *aac(6')-Ie+aph(2'')* and *aph(3')-IIIa* (100%) were resistant to gentamicin and kanamycin, 19 isolates of them (95%) were resistant to amikacin and tobramycin and 16 (80%) isolates were resistant to netilmicin. There were 4 isolates without harboring *mec*A or any of the three aminoglycoside modifying enzymes genes and they showed resistance to all tested aminoglycosides. The *aac(6')-Ie+aph(2'')* (27 isolates) were more frequently detected than *aph(3')-IIIa* (22 isolates) and both genes were more often present together than single (**Tables 7, 8 & 9**).

Table 3. The aminoglycoside resistance phenotype among all S. aureus isolates.

Phenotypic patterns	No. of S. aureus isolates
Frequency (%)	
K	3 (6.5%)
K &A	2 (4%)
K, A &TOB	4 (8.7%)
K, CN & TOB	3 (6.5%)
K, CN, A & TOB	5 (11%)
K, CN, A, NET & TOB	29 (63%)
Total resistance to aminoglycosides	46

CN: gentamicin , A: amikacin , K: kanamycin , NET: netilmicin , TOB: tobramycin.

Table 4. The distribution of aminoglycoside resistance of *S. aureus* isolates in association with cefoxitin resistance.

S. aureus isolates	No.	CN	A	K	NET	TOB
MRSA	39	34	35	38	28	34
MSSA	9	3	5	8	1	5
Total	48	37	40	46	29	39

MRSA; methicillin resistant S. aureus, MSSA; methicillin sensitive *S. aureus* CN; gentamicin, A; amikacin; K: kanamycin; NET; netilmicin; TOB: tobramycin

Table 5. The frequency of aminoglycoside resistance genes among *S. aureus* isolates.

Aminoglycoside suceptible *S. aureus* No.2	Aminoglycoside resistant *S. aureus* No.46	Type of genes
1	26	*aac*(6')-Ie+aph(2'')
0	22	*aph*(3')-IIIa
0	1	*ant*(4')-Ia
1	17	Total no.

Table 6. The distribution of genes encoding aminoglycoside modifying enzymes and *mec*A gene in association with cefoxitin resistance.

Resistance genes	MRSA No= 39	MSSA No= 9	Total No= 48 (%)
aac(6')-Ie+aph(2'')	23 (56%)	4 (44.44%)	27 (56.25%)
aph(3')-IIIa	20 (51.28%)	2 (22.22%)	22 (45.83%)
ant(4')-Ia	1 (2.56%)	0 (0%)	1 (2.08%)
*mec*A	34 (87.18%)	9 (100%)	43 (89.58%)

MRSA: Methicillin resistant *S. aureus*, MSSA: Methicillin sensitive S. aureus

Table 7. The correlation between *aac*(6')-Ie+aph(2'') gene and *mec*A gene in S. aureus isolates.

P value	PCR results for *mec*A		PCR results for aac(6')-*Ie+aph*(2'')
	No. of negative	No. of positive	
0.007	5	16	21 (negative)
	0	27	27 (positive)
	5	43	Total = 48

Table 8. The correlation between *aph*(3')-IIIa gene and *mec*A gene in *S. aureus* isolates.

P value	PCR results for *mec*A		PCR results for *aph*(3')-IIIa
	No. of negative	No. of positive	
0.030	5	21	26 (negative)
	0	22	22 (positive)
	5	43	Total = 48

Table 9. Presence of *mec*A gene and genes coding for aminoglycoside resistance and observed phenotypic aminoglycoside resistance.

TOB	NET	K	A	CN	No %	Genotype mec A/aac/aph/ant
19	16	20	19	20	20	+/+/+/-
6	3	6	3	5	7	+/+/-/-
1	0	2	2	0	2	+/-/+/-
1	1	1	1	1	1	-/-/-/+
8	5	13	10	7	14	+/-/-/-
0	0	0	0	0	0	+/+/-/+
0	0	0	0	0	0	+/+/+/+
0	0	0	0	0	0	+/-/+/+
4	4	4	4	4	4	-/-/-/-

Discussion

Multi-drug resistance is frequently observed among staphylococci clinical isolates worldwide. Therefore, this pathogen is of greatest concern because of its potential virulence, its ability to cause a diverse array of life- threatening infections and its capacity to adapt to different environmental conditions (1).

Although culture-based methods are generally reliable for detecting oxacillin-resistant staphylococci. The detection of *mec*A gene by PCR assay is considered as the gold standard. In particular, multiplex PCR assay that simultaneously detects several genes in a single reaction has the advantage of identifying genotypic resistance for several antibiotics more rapidly and reliably than other methods. Several studies have used multiplex PCR for the detection of genes encoding oxacillin-resistance in staphylococcal isolates (9, 13, 14).

This study showed that 6/48 *S. aureus* isolates were negative by the slide coagulase test, but were positive by tube coagulase test and they were positive. The rate of MRSA isolates was 81.25% using cefoxitin disc. This result was higher than that obtained by Schmitz et al. 1999 (7) (27.7%), Kim et al., 2002 (15) (64%) and Choi et al. 2003 (12) (53%). These different rates of MRSA may be attributed to variations of patient populations, the biological characteristics of the *S. aureus* strains.

The present study found that cefoxitin disc diffusion method and presence of *mec*A gene were not identical in 9 of *S. aureus* isolates. which were cefoxitin susceptible/*mec*A positive and 5 *S. aureus* isolates which were cefoxitin resistant/*mec*A negative. In the case of the 9 cefoxitin susceptible/*mec*A positive isolates, certain auxiliary genes such as *fem*A, *mec*R and genes encoding β-lactamase plasmid may contribute in the control of its expression (12), whereas the 5 cefoxitin resistant/ *mec*A negative isolates, non-PBP2a dependent mechanisms such as hyperproduction of β-lactamases and alteration of PBP types which may control the expression of resistance (16, 17). Mechanisms of oxacillin resistance other than *mec*A are rare and include a novel *mec*A homologue, *mec*C which can't be detected by tests used for detection of *mec*A or PBP 2a (6, 18). Therefore, cefoxitin disc diffusion test may give false results. Detection of *mec*A gene is now considered the gold standard method, mainly because phenotypic method using cefoxitin may be difficult to interpret and some isolates do not express *mec*A unless selective pressure via antibiotic

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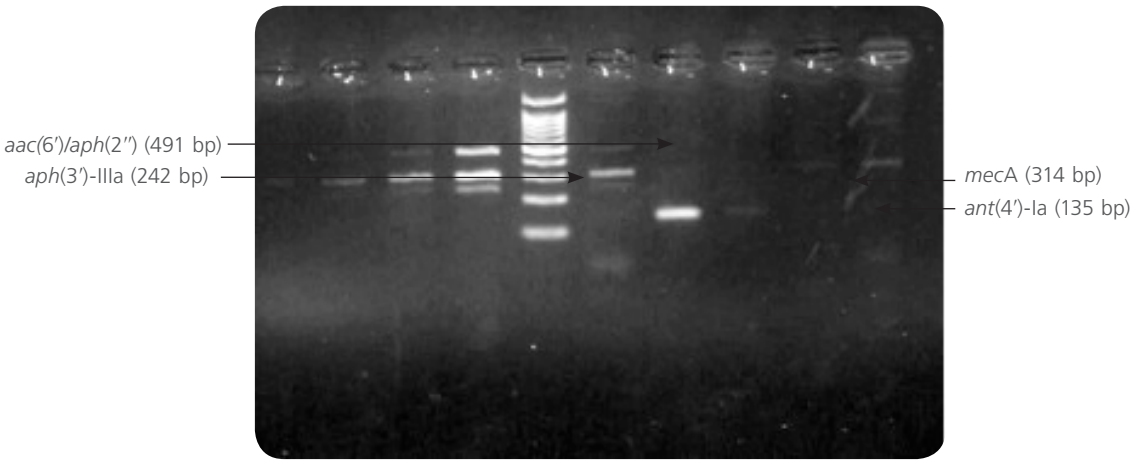

Figure 1. Demonstration of *mec*A gene and the 3 AME genes determined with multiplex PCR in agarose gel. M; molecular size marker, 491 bp band*; aac(6')/aph(2'')* gene; 314 bp band; *mec*A gene; 242 bp band*; aph(3')-IIIa* gene, 135 bp band; *ant(4')-Ia* gene. 15-23 test isolates.

treatment is applied (6). In addition, This study demonstrated discrepancy between the results of oxacillin and cefoxitin disk diffusion method in the form of 2 oxacillin resistant/cefoxitin susceptible isolates but non of these isolates were *mec*A positive by PCR method; similar results was occurred in other studies (19, 20) .

Aminoglycoside resistance has been reported to be common in *S. aureus* isolated from various countries, especially in developing countries (21). In this study, 96% of the 48 *S. aureus* isolates were resistant to at least one of the tested aminoglycoside. The highest staphylococcal resistance was to kanamycin (96%), while the lowest resistance was to netilmicin (60%). This result may be explained by the fact that netilmicin is seldom prescribed in our hospitals. Partial similar results were reported by Hauchild et al., 2008 (22), who found that 97.8% of the 45 staphylococcal isolates were resistant to kanamycin while all isolates were susceptible to netilmicin.

Among staphylococci, the most frequently encountered AME is the bifunctional enzyme 6′-N-acetyltransferase- 2″-O-phosphotransferase [AAC(6′)-APH (2″)], encoded by the *aac*(6′)-Ie-*aph*(2″) gene (7). This bifunctional enzyme inactivates a broad range of clinically useful aminoglycosides by acetylation of an aminogroup and phosphorylation of a hydroxyl group. Additional enzymes such as 4′-O-adenyltransferase I [ANT(4′)-I] is encoded by *ant*(4′)-*Ia* gene. Finally, 3′-O-phosphotransferase III [APH(3′)-III] is encoded by *aph*(3′)-*IIIa* (7, 13, 23).

In our study, the frequency of the genes encoding AMEs, *aac(6′)-Ie+aph(2′′)* encoding the bifunctional enzyme AAC(6′)-Ie+APH (2′′) was the most common (56.5%), followed by the *aph(3′)-IIIa* encoding the APH(3′)-IIIa enzyme (48%) and the *ant(4′)-Ia* gene encoding for the ANT(4′)-Ia enzyme (2%). Similar results were reported by Fatholahzadeha et al., 2009 (24) who found that the most prevalent AME genes were *aac(6′)-Ie+aph(2′′)* (83%) and *aph(3′)-IIIa* (71%) and the least frequent AME gene among MRSA isolates was *ant(4′)-Ia* gene (26%). Similarly, Choi et al., 2003 (12) reported that the gene encoding the bifunctional enzyme was the most frequently encountered gene. This may be explained by the fact that the gene for this bifunctional enzyme is part of a composite transposon Tn*4001,* which is widely distributed in both *S.aureus* and coagulase negative staphylococci that facilitated its rapid dissemination in the presence of selective antibiotic pressure (8). In another study carried out by Schmitz et al. 1999 (7)., the *aac(6′)-Ie+ aph(2″)*, *ant(4′)-Ia* and *aph(3′)-IIIa* were found in a prevalence of 68%, 48% and 14%, respectively of staphylococci resistant to at least one aminoglycoside throughout Europe and the prevalence of *ant (4′)-Ia* and *aph(3′)-IIIa* genes was significantly greater than that reported in previous European studies. Schmitz et al. 1999 (7) explained the change in the prevalence of genes encoding AME due to changes in antibiotic policy and the type of used aminoglycoside, the introduction and consequent inter-hospital spread of resistant strains, especially MRSA or the possibility that these resistance genes could be originated from an environmental source. On the other hand, the study conducted by Ida et al., 2001 (25) in Japan showed higher prevalence of *ant (4′)-Ia* than that of the other two genes. Similar results were also reported by Hauschild et al., 2008 (22). Also, the study conducted by Yadegar et al., 2009 (21),showed that *ant(4′)-Ia* was the most frequent gene (58%), followed by *aac(6′)-Ie+ aph (2″)* and *aph(3′)-IIIa* genes were found in 46% and 6% of the isolates, respectively.

In our study, coexistence of *aac(6')-Ie+aph(2'')* with *aph(3')-IIIa* was seen in 41.66% of isolates. Higher results (71%) were recorded by Fatholahzadeha et al., 2009 (25). This study revealed no significant agreement between presence of *mec*A gene and *aac(6')-Ie+aph(2'')* (62.8%) and *aph(3')-IIIa* (51.2%). This result was similar to the findings of Ardic et al., 2006 (26) which reporting corresponding frequencies for *aac(6')-Ie+aph(2'')* (66%) but rather lower frequency for *aph(3')-IIIa* (8%). A study conducted by Schmitz et al. 1999 (7). reported the following frequencies: 67%, and 20% for *aac(6')-Ie+aph(2'')* and *aph(3')-III* ,respectively, and similar results was reported by Campoccia et al., 2008 (27). Overall, it seems that the prevalence of *aac(6')-Ie+aph(2'')* among methicillin-resistant staphylococci is rather consistent in most studies due to the adjacent locations of *mec*A and of AME encoding genes (26). The detection of resistance genes in antibiotic susceptible *S.aureus* isolates may be due to the fact that AME of these strains display lower enzymatic activity (21,23). However, the study of Ida et al. 2001 (25), reported that all isolates carrying the *aac(6')-Ie+ aph(2")* gene were resistant to gentamicin. Therefore, they were able to detect the production of the bifunctional enzyme AAC(6')-Ie + APH(2") by susceptibility testing with gentamicin. Similar results were reported by Yadegar et al., 2009 (21) who found that all isolates harboring the *aac(6')-Ie+ aph(2")* gene were resistant to gentamicin.

The present study revealed that the gene for the bifunctional enzyme was absent in 12 gentamicin-resistant isolates. Udo and Dashti, 2000 (28) reported also that this gene was detected in all gentamicin-resistant isolates except two. In both isolates this gene could not be detected by PCR and dot blot hybridization. Such observation could be explained by the presence of another variant of the enzyme that could not be detected (23). The presence of variant gene that cannot be detected within the primer or that new aminoglycoside resistance genes are circulating within the *S. aureus* population as explained by Hauschild et al. 2008 (22). This may be also attributed to loss of permeability and ribosomal alteration that may mediate resistance.

It is noteworthy to mention that our *S. aureus* 17 isolates indicated phenotypic resistance to the tested aminoglycosides, none of the three genes encoding the AMEs was detected in these isolates. Similar results were reported by Hauschild et al. 2008 (23) who found that from a total of 45 isolates demonstrating phenotypic resistance to one of tested aminoglycosides, 10 isolates did not harbor the tested genes encoding AMEs. This finding could be due to a new aminoglycoside resistant genes which were horizontally transferred within the *S. aureus* population. Magnet and Blanchard, 2005 (29) reported an explanation for the absence of genes encoding AMEs in resistant *S.aureus* strains most probably due to the presence of other resistance mechanisms like modification of the target by mutation in genes encoding ribosomal proteins, alteration of membrane permeability or active efflux of the drug.

This study indicates that there was a high prevalence of *mec*A and aminoglycoside resistance genes among *S. aureus* isolated from surgical I.C.Us in Kasr Al-Ainy hospitals, Cairo University. kanamycin resistance was the most predominant aminoglycoside resistance among *S. aureus* isolates, and *aac(6')-Ie+aph(2'')* was the most predominant aminoglycoside resistance gene followed by *aph(3')-IIIa* gene. We conclude also that *mec*A gene was not detected all cefoxitin resistant *S. aureus* isolates; therefore, cefoxitin screening test is not sufficient to detect MRSA. A more sensitive phenotypic method using oxacillin minimal inhibitory concentration (MIC) is recommended to evaluate the susceptibility of cefoxitin disc diffusion method for MRSA detection. Periodic and rapid detection of aminoglycoside resistance and the corresponding genes in MRSA isolates is needed to avoid using costly and high toxicity antibiotics. It is important to control development of antibiotic resistant by monitoring potential developing of new aminoglycoside resistant genes that may be produced within *S. aureus* population. This procedure will help to establish effective antibiotic therapies and prevent nosocomial infection as well as environmental spread of resistant strains. A more prudent use of aminoglycosides is required since aminoglycosides resistance is closely associated with methicillin resistance. Rapid detection of antibiotic resistance by PCR, particularly MRSA associated with aminoglycosides resistance will reduce the misuse of empirical treatment with broad spectrum antibiotics.

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